STUDIES ON CANINE VIRAL ENTERITIS

Principal Investigators : Markpol Tingpalapong, DVM, LL.B.
Richard E. Whitmire, LTC, VC
Leonard N. Binn, Ph.D*
Michael A. Ussery, Ph.D
Sukhum Sujarit, DVM, LTC, RTA**
Somsakdi Ruksil, DVM, CPT, RTA**

Associate Investigators : Robert A. Graham, CPT, VC
Donald S. Burke, MAJ, MC
Somkiat Changnak, B.Sc.
Aree Boriharnyanakett, B.Sc.

OBJECTIVES :

1. To identify and describe the etiologic agent producing a severe, bloody, often fatal diarrheal viral disease of canines in the military working dogs at the Royal Thai Army National War Dog Center in Pak Chong and the Royal Thai Navy Military Dog units at Sattaheep.

2. To produce Canine Viral Enteritis in susceptible, weanling dogs.

3. To study the serologic response of Military Working Dogs to killed Feline Panleukopenia Vaccine under field conditions.

BACKGROUND : During 1979, a severe outbreak of Canine Viral Enteritis (CVE) caused by a canine parvo-like virus was detected in the Military Working Dog Center (MWDC), Pakchong, Nakornrajsima, Thailand. The outbreak resulted in the death of 187 of 244 (76.6%) puppies that were born in MWDC and 48 of 111 (43.2%) puppies purchased from local sources.

Phase I of the study identified canine parvovirus as the etiologic agent involved in the outbreak of fatal diarrhea in dogs at the MWDC, the Royal Thai Navy Military Dog unit at Sattaheep, and privately-owned dogs in Bangkok.(1)

Phase II of the study involved the attempt to experimentally reproduce the disease in susceptible young, weanling dogs. In this study, 12 susceptible dogs were infected with parvovirus isolated from the stool of a positive dog from the MWDC. The infected stool was positive by electromicroscopy and was given orally in a 2% suspension buffered with 1% phosphate buffer solution and containing 1% Bovine Serum albumin. Two control dogs were given only the phosphate buffered solution.

Phase III of the study was to follow the serologic response of military Working Dogs to killed Feline Panleukopenia Vaccine given under field conditions.

---

* Division of Veterinary Medicine, WRAIR, WRAMC, Washington, D.C. 20012

** Military Working Dog Center, Pakchong, Nakornrajsima, Thailand.

This vaccine was chosen because at the time, it was the only product on the market that had any chance of offering some protection to susceptible dogs. (Feline panleukopenia is a severe, often fatal disease in cats caused by a feline parvovirus).

Experimental inoculation with Feline Panleukopenia Virus Vaccine protected dogs against challenge with Canine Parvolike-virus.[2] In an attempt to protect dogs from Canine Viral Enteritis, MWDC purchased 200 doses of Feline Panleukopenia vaccine (FPLV)[a]. This is a commercially available killed vaccine for immunization of cats against Feline panleukopenia.

METHODS :

Specimens : Ninety-four dogs were involved in this third phase of the study. Seventy-eight were given two inoculations of FPLV one month apart and 16 dogs were not vaccinated and served as controls. Serum specimens were collected the day before vaccination, and at one month, three months and six months after vaccination.

Serology : Sera specimens (0.1 ml each) were heat inactivated at 56°C for 30 minutes and placed in 10 ml of cold acetone[b] for 5 minutes in an ice bath with periodic shaking. Then specimens were centrifuged in cold at 1800 rpm for 5 minutes. Following centrifugations, the supernatant acetone was poured off and the precipitate re-extracted with the same volume of acetone. After recentrifugation and removal of acetone the extracted serum is left at room temperature overnight. The dried material is dissolved in 0.5 ml PBS (0.01M pH 5.8 isotonic solution).

All sera are adsorbed with cells prior to use in tests. To each serum is added 0.1 ml of packed washed rhesus erythrocytes, and left in an ice-box for 20 minutes with occasional shaking, after which the tubes are centrifuged at 1800 rpm for 10 minutes in the cold. The supernatant fluids are now ready for use in the HI test.

HA antigen preparation - Virus suspension (from Dog No. 1815) was inoculated into Canine A-72 cell culture.[c] There are 100,000 original A-72 cells in one-milliliter of L-15[d] media in each 15 ml screw cap tubes. The cells that exhibited CPE on day three were harvested and disrupted by three cycles of freezing, thawing and sonicating. After centrifugation at 500 g for 15 minutes, supernatant fluid was aliquoted at 0.5 ml and kept frozen at -70°C.

---

a = Killed Virus, Feline cell origin, Norden, Lincoln NE 68501 USA

b = Matheson Coleman & Bell, Norwood, Ohio, 45212 USA

c = Division of Veterinary Medicine, WRAIR, WRAMC, Washington, D.C. 20012

d = Leiboitz's L-15 Medium, Powdered Tissue Culture Medium, Cat No. 430-1300 Grand Island, New York 14072 USA

RESULTS :

Phase I of the study has been written up and submitted to the publisher.[1]

Phase II of the study dealing with the experimental production of Canine Viral Enteritis is still in the process of laboratory analysis. Clinical illness was produced in some of the dogs on study and stool, sera, and tissue specimens are now stored in Revcos at -70°C or in formalin. Funds for this study have been curtailed and further analysis will depend on the availability of funds and manpower.

Phase III of the study involving the serologic response to FPLV under field conditions is currently being analyzed. The sera have been tested for HAI and the preliminary results are found in Table 1.

REFERENCES :

1. Tingpalapong, Markpol, R.E. Whitmire, D. Watts, L. Binn, et al. An Epizootic of Viral Enteritis in Dogs of Thailand, submitted to The American Journal of Veterinary Research.

2. Appel, M.J.G., F.W. Scott, L.E. Carmichael, 1979. Isolation and Immunization Studies of a Canine Parvo-like Virus from Dogs with Haemorrhagic Enteritis. Vet. Res. 105: 156-159.

Table 1. Haemagglutination-inhibition antibody titers in dogs after vaccination with killed feline Panleukopenia Virus.

Dog Number	Age	Pre-Vaccination	One month after one Vaccination	Three months after first Vaccination	Six months after first Vaccination
Ben	>1 yr	160	80	80	40
Mon	>1 yr	160	320	160	40
A3111	<1 yr	320	320	320	80
B3106	<1 yr	160	160	160	-
B3110	<1 yr	320	640	1280	-
D38	>1 yr	320	160	160	80
D43	>1 yr	640	320	640	320
D45	>1 yr	320	640	640	640
D5922	<1 yr	160	320	160	640
F3009	<1 yr	160	320	80	80
F3010	<1 yr	160	160	160	80
F3011	<1 yr	320	320	320	320
FM0448	<1 yr	320	320	320	40
H9502	<1 yr	320	320	320	20
HA0519	>1 yr	640	320	20	-
HEE1502	>1 yr	80	80	80	80
HM1818	>1 yr	40	80	80	40
HM1819	>1 yr	160	160	80	640
LA2509	>1 yr	320	320	320	320
LB1200	>1 yr	80	320	320	160
LB1272	>1 yr	320	320	320	320
LB1349	>1 yr	80	80	80	80
LB1446	>1 yr	80	160	80	320
LB1483	>1 yr	320	20	10	-
LB1501	>1 yr	160	320	320	160
LB3153	>1 yr	640	1280	640	640
M3015	<1 yr	640	320	320	160

Table 1. (continued)

Dog Number	Age	Pre-Vaccination	One month after one Vaccination	Three months after first Vaccination	Six months after first Vaccination
ME0803	>1 yr	320	320	160	320
ME1170	>1 yr	320	1280	640	1280
ME3022	>1 yr	640	640	640	320
ME3068	>1 yr	320	640	640	640
ME3308	>1 yr	320	320	160	640
MF0865	>1 yr	160	320	160	320
MF1040	>1 yr	320	160	320	160
MF1267	>1 yr	320	320	320	320
NT1062	>1 yr	320	320	320	80
PL0440	>1 yr	320	160	160	80
R3004	<1 yr	320	160	160	80
R3005	<1 yr	80	80	80	80
R3006	<1 yr	160	80	80	20
R3008	<1 yr	320	5120	640	80
R3011	<1 yr	640	320	320	160
R3012	<1 yr	80	80	160	80
R3015	<1 yr	640	320	80	40
T0122	<1 yr	320	640	640	640
T0222	<1 yr	40	160	80	40
T0322	<1 yr	80	320	320	320
X3073	<1 yr	160	160	320	160
0121	>1 yr	160	80	40	40
0223	<1 yr	160	320	160	160
0423	<1 yr	160	80	40	-
1718	>1 yr	320	320	80	320
2022	>1 yr	20	40	80	320
2821	>1 yr	320	320	640	-
3222	>1 yr	80	320	320	80

Table 1. (continued)

Dog Number	Age	Pre-Vaccination	One month after one Vaccination	Three months after first Vaccination	Six months after first Vaccination
3380	>1 yr	320	320	320	320
3819	>1 yr	320	320	320	80
4622	<1 yr	1280	320	-	80
4718	>1 yr	40	40	80	80
4819	>1 yr	80	160	160	80
4822	<1 yr	640	160	40	-
4922	<1 yr	80	80	80	40
5218	>1 yr	40	80	80	80
5317	>1 yr	80	80	80	80
5422	<1 yr	40	40	40	40
5622	<1 yr	80	160	80	80
5722	<1 yr	40	80	80	20
5922	<1 yr	80	160	80	-
6022	≤1 yr	80	80	640	20
7819	>1 yr	160	80	80	160
8618	>1 yr	80	80	80	40
8717	>1 yr	320	320	320	640
8718	>1 yr	640	320	80	-
11119	>1 yr	320	320	320	80
11519	>1 yr	160	320	40	40
12419	>1 yr	320	640	640	320
95257	>1 yr	320	640	80	640
331145	>1 yr	640	640	640	640
IA2155*	>1 yr	320	80	40	80
IA2156*	>1 yr	80	40	40	20
LB1333*	≤1 yr	160	160	80	40
LB3228*	>1 yr	320	320	320	320
LK2529*	<1 yr	320	80	320	80

Table 1. (continued)

Dog Number	Age	Pre-Vaccination	One month after one Vaccination	Three months after first Vaccination	Six months after first Vaccination
V1001*	>1 yr	320	320	320	-
0519*	>1 yr	320	320	320	320
2521*	<1 yr	640	320	320	160
2921*	>1 yr	80	80	80	80
4017*	>1 yr	320	320	320	320
4221*	>1 yr	320	1280	1280	-
5617*	>1 yr	320	320	160	80
5920*	>1 yr	80	80	320	10
6720*	>1 yr	80	160	40	-
6818*	>1 yr	320	160	640	-
12919*	>1 yr	160	80	80	-

Experimental dogs were vaccinated twice with commercial killed feline panleukopenia vaccine, a dose for cat each time intramusculary, one month interval.

* Control dog.